(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 May 2003 (22.05.2003)

PCT

(10) International Publication Number
WO 03/042364 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US02/35901

(22) International Filing Date:
8 November 2002 (08.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/338,136 9 November 2001 (09.11.2001) US

(71) Applicant *(for all designated States except US)*: **DANA-FARBER CANCER INSTITUTE, INC.** [US/US]; 44 Binney Street, Boston, MA 02115 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **POLYAK, Kornelia** [HU/US]; 1856 Beacon Street, #6F, Brookline, MA 02445-1946 (US). **PANKAJ, Seth** [IN/US]; Unit #8, 109 Queensberry Street, Boston, MA 02215-4708 (US).

(74) Agent: **FRASER, Janis, K.**; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR IDENTIFYING, ISOLATING, AND CONTROLLING THE GROWTH OF ESTROGEN-RESPONSIVE CELLS

(57) Abstract: The invention features reporter constructs and reporter vectors useful for the identification and isolation of estrogen-responsive cells. The invention also embraces methods of inhibiting the proliferation or survival of estrogen-responsive breast cancer cells.

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Fig. 3B is a photograph of northern blots showing the expression of lipocalin 2, S100A2, and β-actin RNA in mammary glands from (i) virgin female mice ("V"); (ii) ovariectomized virgin mice ("Ov"); (iii) virgin mice that had been ovariectomized and then treated for 6 hours with estrogen ("Ov+E6h"); (iv) virgin mice that had been ovariectomized and then treated for 12 hours with estrogen ("Ov+E12h"); and (v) lactating mice ("L").

Fig. 3C is a series of photomicrographs of histological sections of normal human breast tissue analyzed by (i) mRNA in situ hybridization with digotonin-labeled antisense and sense lipocalin 2 and S100A2 riboprobes; and (ii) immunohistochemical staining with an antibody specific for estrogen receptor α ("ERα IHC"). The immunohistochemical analyses were carried out on tissue sections immediately adjacent to those used for the respective in situ mRNA hybridization analyses.

Fig. 3D is a bar graph showing the numbers of colonies that were obtained after drug (hygromycin) selection (for 2 weeks) of estrogen receptor ("ER") expressing ("Positive") MCF-7 and T47D breast cancer cells, estrogen receptor non-expressing ("Negative") BT549 and MTA-MB-435S breast cancer cells, and estrogen receptor non-expressing ("Negative") MCF10A normal immortalized mammary epithelial cells that had all been transfected with either a control expression vector ("CEP") or an expression vector containing cDNA encoding human lipocalin 2 fused at its C-terminus to a double hemagglutinin tag ("LIPO"). The experiments were carried out in T25 tissue culture flasks and the data are expressed as the number of "Colonies/T25 flask".

Fig. 4 is a bar graph showing the number of colonies that were obtained after culturing estrogen receptor expressing 747D breast cancer cells and estrogen receptor non-expressing MCF10A normal immortalized mammary epithelial cells with conditioned medium from CHO cells recombinantly expressing either GFP ("GFP") or lipocalin 2 ("LIPO"). The experiments were carried out in the wells of 6-well tissue culture plates. Data, which are expressed as "Colonies/well", from experiments carried out in medium supplemented with 5% fetal bovine serum ("5% FBS") or 0.2% fetal bovine serum ("0.2% FBS") are shown.

Fig. 5A is a depiction of the nucleotide sequence (SEQ ID NO:10) of the reporter construct depicted in Fig. 1A. The five cassettes containing the five different EREs are shown in bold and are underlined. The rat progesterone receptor distal promoter sequence is shown in bold italics. Sequence (derived from the pEGFPN1 vector) containing the GFP coding sequence is in plain